

Determination of the Best Harvesting Times to Obtain Maximum Dry Herbage, Essential Oil and Thymol Yield in Garden Thyme (*Thymus Vulgaris* L.)

Ahmad Reza Golparvar

Department of Agronomy and Plant Breeding, Khorasgan (Isfahan) Branch,
Islamic Azad University, Isfahan, Iran

Abstract-Thyme (*Thymus vulgaris*) is widely used in pharmacy, cosmetics and Food industries of developed countries; it is a strong disinfectant with antioxidant. Randomized complete blocks design was used to determine the effects of harvesting times on herbage yield and quality/quantity of oil in Garden thyme. This study was conducted in research farm of Khorasgan branch of Islamic Azad University in 2010-2012 years. Plants were harvested in five harvesting times, i.e. in the vegetative stage, beginning of blooming, 50% blooming, full blooming and fruit set stages. Oils were extracted by hydrodistillation. Thymol percentage was determined by GC/MS. Results showed the significant effects of harvesting times on essence and thymol yield and percentage. Mean comparison showed that beginning of blooming has significantly the highest essence efficiency (2.51%). Although, 50% blooming stage showed the highest fresh and dry herbage as well as essence and thymol yield. In conclusion, it is recommended to harvest this plant at 50% blooming to gain the highest essence and thymol yield and dry herbage in *Thymus vulgaris* L.

Keywords-Thyme; harvesting times; dry herbage; essential oil and thymol yield

I. INTRODUCTION

Garden thyme (*Thymus vulgaris* L.) is belonged to mint family (Lamiaceae). The origin of this plant is Mediterranean region, North of America, and some parts of Asia (Stahl-Biskup and Saez, 2002). Thyme has a wooden plant, short, perennial and Grey colored with C3 metabolism system, which will be 30-50 cm tall depending the climate of growth region (Stahl-Biskup, 1991; Ozguven and Tansi, 1998).

At present time, this plant is cultivated in large scale in Iran. Evidently, thyme continues to command an important place in expanding world market. Thyme volatile phenolic oil has been reported to be among the top 10 essential oils (Letchamo and Gosselin, 1996), showing antibacterial, antimycotic, antioxidative, natural food preservative, and mammalian age delaying properties (Letchamo and Gosselin, 1996; Jackson and Hay, 1994).

The biosynthesis of secondary metabolites, although controlled genetically, is affected strongly by environmental influences. Agricultural factors have a critical effect on quantitative and qualitative characteristics of thyme, which finally result in plant growth and yield increment. The harvesting times can be very effective factor in this area

(Mirahmadi et al., 2010; Omidbaigi et al., 2005; Golparvar et al., 2011).

In Sefidkon *et al.* (2009) study about effects of harvest stages and various methods of hydrodistillation on essence efficiency of Garden thyme, harvesting times had significant effect on essence efficiency. Mean comparison results showed that the highest efficiency belonged to beginning of blooming (1.18%) and vegetative phase had the lowest (0.86 %). In other research, the highest efficiency belonged to full blooming (1.71%) whereas fruit set stage (0.18%) had the lowest (Hornok, 1991). Also some studies showed that the highest herbage yield and essence of Garden thyme were obtained in lower elevations and in full blooming stage (Hudaib and Aburjai, 2007; Ozguven and Tansi; 1998).

The effect of seasonal regime on amount and essence components of Garden thyme was studied in New Zealand and the highest essence yield was obtained in December (22.8 lit/ha) after blooming. Essence components showed also lots of changes in a 13 months period. The highest level of phenol compounds, i.e. thymol and carvacrol was observed in summer after blooming stage (McGimpsey *et al.*, 2006). Jordan *et al.* (2006) in a study about effects of harvesting times concluded also that in hymalian thyme (*Thymus hyemalis* Lange.) the highest percentage of thymol and carvacrol were obtained in beginning of blooming. Nejad-Ebrahimi et al. (2008) find that the lowest essence percentage and the highest percentage of carvacrol were obtained in vegetative phase.

Omidbaigi *et al.* (2005) with studying effect of various harvest times on quality and quantity of thyme (*Thymus critriodorus*(pres.) Schreb) showed that the highest essence yield (2.21%) was obtained in beginning of blooming. The most extent compound in essence was geraniul which its lowest (54.21%) and highest (72.48%) amounts in essence were obtained in seed set and vegetative stages, respectively. Khorshidi *et al.* (2010) Investigated four various stages in two region to assess the effect of climate and harvesting times on essence percentage of Denaian thyme (*Thymus daenensis* Celak.). Results showed higher essence percentage in full blooming stage in both regions (3.4% and 2.93% in Malaayer and Hamedan, respectively). Mirahmadi *et al.* (2010) reported that the highest mean of essence percentage in *Thymus daenensis* Celak. was obtained in full blooming stage (3.4%) whereas fruit and seed set stages had the lowest (2.17%).

Gharabaghian thyme (*Thymus fedtschenkoi*) showed similar results (full blooming: 2.94 %; fruit and seed setting: 0.66%).

Therefore, this study was achieved to assess the effect of harvesting times on herbage yield and quality/quantity of oil in thyme as well as to determine the best harvesting time in Garden thyme (*Thymus vulgaris* L.).

II. MATERIALS AND METHODS

The research was achieved in research farm of Khorasan (Isfahan) branch of Islamic Azad University in two years; 2010-2011 (first year) and 2011-2012 (second year) using a randomized complete blocks design with three replications. Harvesting times i.e. in the vegetative stage, beginning of blooming, 50% blooming, full blooming and fruit set stages were considered as treatments. Seeds of *Thymus vulgaris* L. were sown in green house at 2010 February. Small seeds were mixed with gravel to facility and uniformity in sowing (one part seeds plus two part gravel). Seeds were sown in 0.5 cm depth. The amount of used seeds was 0.8 to 1 gram per square meter. There was no disease during growth in green house. Seedlings transplanting was done at April 2010.

The research farm is located in east of Isfahan (32° 38'N 51°47'E) with 1550 m elevation. Region climate was dry or cold dry according to Demartin and ambergay methods, respectively. Mean annual precipitation is 140.63 mm and mean temperature of region is 16.8°C. Maximum temperature in July was 46.3 °C and minimum in January was 14.33°C. Evapotranspiration of this region is 1802.31 mm per year. Soil texture was silty-loam with 1.6% of organic carbon, 0.02% of nitrogen, 20 p.p.m of available phosphorus, 504 p.p.m of available potassium, pH=7.5, acidity of 7.6 and 3.1 mmohs/cm electrical conductivity in 0 to 30 cm depth.

Rooted cuttings had been planted in rows 50 cm apart with inter-row spacings of 20 cm apart. Experimental units consist of four rows of 3m length. Irrigation was done every 4-6 days using furrow method. Weed control was conducted during growing season. Any pest was observed on thyme plants.

Traits i.e. fresh and dry herbage (kg/ha), essence percentage (essence weight obtained from 100 gr. dry matter), essence yield (dry herbage × essence percentage (kg/ha)), thymol percentage and thymol yield (thymol percentage × essence yield (kg/ha)) were measured for every experimental unit.

Sampling was done from middle rows of each plot by eliminating border effects. Fresh herbage was recorded as biomass wet weight. These samples were dried in shaded area in room temperature with appropriate ventilation for four days and then measured as dry herbage. Wooden parts were separated and 100 gram of dried biomass was prepared to essence hydrodistillation.

Oils were extracted by hydrodistillation for 3 h, of the aerial parts using Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and kept at -4 °C until it was analyzed. Qualitative and quantitative analyses of oils were performed by Shimadzu gas chromatography model 15A, equipped with a FID detector and fused silica capillary column (OV-101, 25m × 0.2 mm). GC analytical conditions

were: injector temperature: 230 °C, oven temperature: 175 °C (isothermal), and detector temperature: 230 °C. The percentage of the thymol is computed from GC (FID) peak areas with using the area normalization method (Shibamoto, 1987).

Analysis of variance of data obtained in the second year (2011-2012) was done based on randomized complete blocks design model. Then existence of significant difference between means of harvesting times were evaluated by Duncan's new multiple range test (DNMRT) at 5% probability level. SPSS₁₆ program was used to statistical analysis.

III. RESULTS AND DISCUSSION

Analysis of variance (table 1) showed the significant effect of different harvesting times on essence percentage, essence yield, thymol percentage and thymol yield. Mean comparison of treatments (figure 1) showed that fresh herbage of vegetative phase had significant difference with the other stages. The highest fresh herbage (8830 kg/ha) resulted in 50% blooming and the lowest (4530 kg/ha) obtained from vegetative phase (Figure 1). Naghdibadi *et al.* (2004) found that various stages of growth had significant effect on wet weight. Mean comparison among harvesting times showed that the highest fresh weight (17.70 ton/ha) was obtained in flowering initiation whereas full flowering had the lowest (15.56 ton/ha).

The highest dry herbage (2594.09 kg/ha) was related to 50% blooming and vegetative phase showed the lowest (1608.39 kg/ha). But differences among the means of different harvesting times were not significant for dry herbage (Figure1). According to Naghdibadi *et al.* reports (2004), effects of various harvest times were not significant on dry herbage and the highest quantity of dry herbage (5.63 ton/ha) was resulted in seed setting stage whereas flowering initiation phase had the lowest (5.54 ton/ha). Ozguven and Tansi (1998) showed that various harvesting times have significant effect on dry biomass weight and the highest dry matter was obtained in seed setting phase.

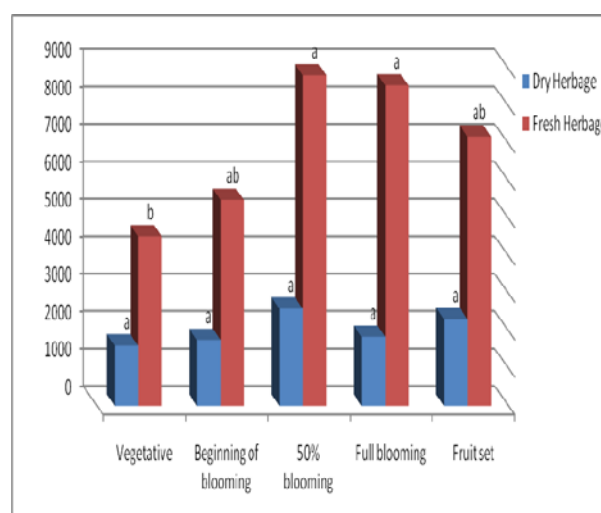


Figure1 - Mean comparison of harvesting times for fresh and dry herbage (Kg/ha) in Garden thyme (In each trait, means with the same letter (s) haven't significant difference according to Duncan's new multiple range test)

TABLE 1 ANALYSIS OF VARIANCE OF HARVESTING TIMES FOR PERCENTAGE AND YIELD OF ESSENCE AND THYMOL IN GARDEN THYME (*THYMUS VULGARIS* L.)

Source of variance	df	Fresh herbage	Dry herbage	Essence percentage	Essence yield	Thymol percentage	Thymol Yield
block	2	140.94 ^{ns}	3.54 ^{ns}	0.0004 ^{ns}	0.19 ^{ns}	1.42 ^{ns}	0.41 [*]
treatment	4	452.16 ^{ns}	82.84 ^{ns}	0.508 ^{**}	451.20 ^{**}	810.63 ^{**}	132.30 ^{**}
error	8	132.03	61.34	0.0002	0.06	0.93	0.052

ns, * and **: non-significant and significant at 5% and 1% probability levels, respectively.

TABLE 2 MEAN COMPARISON OF HARVESTING TIMES FOR PERCENTAGE AND YIELD OF ESSENCE AND THYMOL IN GARDEN THYME (*THYMUS VULGARIS* L.)

Phenological stage(treatments)	Essence percentage (%)	Essence yield(kg/ha)	Thymol percentage (%)	Thymol yield (kg/ha)
Vegetative	1.43 ^e	23.00 ^e	58.2 ^c	13.39 ^e
beginning of blooming	2.51 ^a	44.18 ^b	43 ^d	18.99 ^d
50% blooming	1.86 ^b	48.25 ^a	60.6 ^b	29.24 ^a
Full blooming	1.62 ^d	29.90 ^d	70.7 ^a	21.14 ^c
Fruit set	1.73 ^c	40.12 ^c	59.1 ^b	23.71 ^b

In each column, means with the same letter (s) haven't significant difference according to Duncan's new multiple range test

Studying the ascending trend of fresh and dry herbage changes (Figure 1) showed that fresh herbage had an obvious increasing from vegetative phase to 50% blooming that plus the length of plant growth it could be because of shiny days with high temperatures, specifically in July and August because days are longer in this period and then radiation is more. In full blooming stage, weight had a little decrease that was probably because of late summer low temperatures (Letchamo and Gosselin, 1996)

Mean comparison of various harvesting times (table 2) showed that the most essence percentage (2.51% volume per weight) was related to beginning of blooming and vegetative phase had the least (1.43 % volume per weight). Also, the most essence yield (48.25 kg/ha) and the least (23 kg/ha) belonged to 50% blooming and vegetative phase, respectively (table 2).

Naghdibadi *et al.* (2004) showed that different harvesting times didn't affect essence yield but significantly affected essence percentage at 5% probability level. Results of mean comparison expressed that the highest essence yield (115 kg/ha) was resulted in beginning of blooming and fruit set stage had the lowest essence yield (101.8 kg/ha). The highest essence efficiency (20.8%) belonged to beginning of blooming whereas full flowering stage had the lowest (1.8%).

Result of Sefidkon and Rahimibidgoli (2002) about *Thymus kotschyanus* Boiss showed that full flowering was the best time to gain the highest essence amount. Nejad-Ebrahimi *et al.* (2008) in their study on *Thymus caramanicus* Jalass observed that the lowest essence was obtained in vegetative phase (before flowering).

Evaluation of the variations happened in essence percentage reveal that this plant has less essence in vegetative

phase (table 2) but after transition to blooming stage will have obvious increase in essence amount, and then with blooming completion, essence amount will reduce obviously that can be because of different external/internal factors. This phenomenon is not only important for essence amount but also is interesting from other aspects like changes in amount of some of its components. Environmental factors like temperature, humidity, light, location, soil and ... are important but this is essential to know that clearing up effects of environment will not reduce the role of genetic factors which may be themselves affected by environment.

Thompson *et al.* (2003) mentioned high temperatures as the reason of higher essence production of Garden thyme. According to results of Cristina Figueiredo *et al.* (2008), high temperatures could limit photosynthesis in *Thymus vulgaris* L. and also with changing absorption of nutrients from soil would sway organic matter production, sugar and amino acids. In this situation plant feels stress and with reduction in activity of primary metabolites cycles activates secondary metabolites (essence) to resist against stress which it increases essence as result. They also mentioned that low temperature was an effective factor in reduction of essence production in Garden thyme.

According to extent reports, Garden thyme in Iran has 0.8 to 2.6% essence. The best harvest time is different in various regions. In this study, the most essence percentage (2.51%) was obtained in flowering start that is in agreement with results of Naghdibadi *et al.* (2004) in Karaj, and also with founds of Sefidkon *et al.* (2009) on Garden thyme, and Omidbaigi *et al.* (2005) on *Thymus citriodoruspers* Scherb. The most thymol percentage (74.8%) was obtained in full blooming but the least (31%) belonged to beginning of blooming (table 2). Also, 50% blooming stage had the most

thymol yield (24.15 kg/ha) whereas vegetative phase had the least (10.12 kg/ha)(table2).

Researches on Garden thyme conducted by Naghdibadi et al. (2004) showed that the highest thymol amount (47.99%) and thymol yield (55.28 kg/ha) were related to flowering initiation. Changes regime of thymol percentage showed that this plant had low thymol in vegetative phase but with passing vegetative phase and entrance to flowering phase, an obvious reduction in thymol amount occurred, then in full flowering, thymol amount increased highly that its reason could be various environmental and genetic factors. Jordan et al. (2006) in research of various harvesting times effects on essence quality and quantity of Himalayan thyme (*Thymus hyemalis*) observed that the highest density of gamma-terpinene (starter of p-cymene synthesis) was related to full flowering but the highest densities of thymol and carvacrol were obtained from full flowering and start of fruit ripening. The highest densities of alcohols, Ketons and steres were obtained in vegetative phase. Sefidkon et al. (2009) observed that thymol amount was increased gradually in Garden thyme from the first of vegetative phase to full flowering.

IV. CONCLUSION

Te results showed that to reach the highest amount of essence in *Thymus vulgaris* L., it is better not to harvest in vegetative phase because the best time for the highest essence amount is beginning of blooming, but for obtaining the highest essence yield, thymol yield, fresh and dry herbage, harvesting in 50% of flowering is better than other harvesting times. Research about aromatic and medicinal plants which are not native of Iran, especially frequently used plants like Garden thyme and evaluating qualitative and quantitative yield of essence and chemical compounds of these species on farm situation is necessary. Planting a medicinal plant will be economical if its secondary metabolites production were in a favorable amount. Considering the daily increasing in thyme use for Pharmacia industries and high request for scientific production, it is recommended to harvest this plant in 50% blooming in Isfahan region (Iran) and similar areas.

ACKNOWLEDGEMENT

This research project has been supported by Islamic Azad University, Khorasgan (Isfahan) branch, Isfahan, Iran. This support is highly appreciated.

REFERENCES

- [1] Cristina Figueiredo A, Barroso JG, Pedro LG, Scheffer JJC (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour. Fragrance. J.*, 23: 213 – 226.
- [2] Golparvar AR, Ghasemi Pirbalouti A, Karimi M (2011). Determination of the effective traits on essence percent and dry flower yield in German chamomile (*Matricaria chamomilla* L.) populations. *J. Medicinal. Plants. Res.*, 5(14): 3242-3246.
- [3] Hornok L (1991). Cultivation and processing of medicinal plants . Academic Publ. Budapest, pp.338.
- [4] Hudaib M, Aburjai T (2007). Volatile components of *Thymus vulgaris* L. from wild - growing and cultivated plants in Jordan. *Flavor. Fragrance. J.*, 22:322–327.
- [5] Jackson SAL, Hay RKM (1994). Characteristics of varieties of thyme (*Thymus vulgaris* L.) for use in the U.K., Oil content, composition and related characters. *Ann. Hort. Sci.*, 69: 275-281.
- [6] Jordan MJ, Martinez RM, Goodner KL, Baldwin EA, Sotomayor A (2006). Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgais* L. essential oils compositions. *Industrial Crops and Products*. 24, 253–263.
- [7] Khorshidi J, Rostaei A, Fakhre Tabatabaei M, Omidbaigi R, Sefidkon F (2010). Effect of climate and harvesting time on essential oil quantity of *Thymus daenensis* Celak. Scientific Conference on Medicinal plant Industry Development in Iran. 28 February & 1 March 2010 Tehran – Iran.
- [8] Letchamo W, Gosselin A (1996). Transpiration, essential oil glands, epicuticular wax and morphology of *Thymus vulgaris* are influenced by light intensity and water supply. *J. Hort. Sci.*, 71:123 – 134.
- [9] McGimpsey JA, Douglas MH, Van Klink JW, Beauregard DA, Perry NB (2006). Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. *Flavour. Fragrance. J.*, 9(6): 347-352.
- [10] Mirahmadi F, Omidbaigi R, Sefidkon F, Rostaei A, Fakhre Tabatabaei M (2010). Compare the quality of essential oil from *Thymus fedtschenkoi* at different stages of plant growth. Scientific Conference on Medicinal plant Industry Development in Iran. 28 February & 1 March 2010 Tehran – Iran.
- [11] Naghdi Badi H, Yazdani D, Ali SM, Nazari F (2004). Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. *Ind. Crops Prod.* 19, 231–236.
- [12] Nejad-Ebrahimi S, Hadian J, Mirjalili MH, Sonboli A, Yousefzadi M (2008). Essential oil composition and antibacterial activity of *Thymus caramanicus* at different phenological stages. *Food Chemistry.*, 110: 927-931.
- [13] Omidbaigi, R., Sefidkon, F., and Hejazi, M. 2005. Essential oil composition of *Thymus-citriodorus* L. cultivated in Iran. *Flavour and Fragrance Journal.*, 20: 227-238
- [14] Ozguven M, Tansi S (1998). Drug Yield and Essential Oil of *Thymus vulgaris* L. as in Influenced by Ecological and Ontogenetical Variation Cukurova University, Tr. J. Agric. Forestry., 22: 537-542.
- [15] Sefidkon F, Rahimi Bidgoli A (2002). Assessment of quantitative and qualitative variation of essence in *Thymus kotschyanus* in plant growth period and different distillation methods. *Iranian. Medicinal. Aromatic. Plants. Res.*, 15: 1-22.
- [16] Sefidkon F, Nikkhah F, Sharifi Ashoorabadi E (2009). The effect of distillation methods and plant growth stages on the essential oil content and composition of *Thymus vulgaris* L. *Iranian. Medicinal. Aromatic. Plants. Res.*, 25(3): 309 – 320.
- [17] Shibamoto T (1987). In: Sandra, P., Bicchi, C. (Eds.), *Capillary Gas Chromatography in Essential Oil Analysis*. Hüthig, Heidelberg, p. 259.
- [18] Stahl-Biskup E (1991). The chemical composition of thymus oil. *J. Essent. Oil. Res.*, 3: 61-82 .
- [19] Stahl-Biskup E, Saez F (2002). *Thyme*. London: Taylor and Francis, pp. 20-42.
- [20] Thompson JD, Chalchat JC, Michet A, Linhart YB, Ehlers B (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J. Chemical. Ecology*. Vol. 29 (4): 858-880.